U.S. Fish & Wildlife Service

California Nevada Fish Health Center
FY2007 Investigational Report:

Lack of disease response in juvenile Upper Klamath Lake suckers (age 0+) to adverse water quality conditions- Pilot study August 2007.

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Summary: Sucker fry (27 – 30 mm standard length, 79% Shortnose suckers) were held for 22 days in A-canal water during August 2007. Water quality data collected from the aquaria system included temperature, ammonia, pH, dissolved oxygen, and specific conductivity. Fry were sampled on a weekly basis for histological analysis as well as for infection by internal bacteria and external parasites. We did not observe disease, tissue abnormalities, or significant mortality in sucker fry exposed for 22 days to adverse water quality that was comparable to extreme levels of ammonia (0.102 mg /L NH_3) and minimal dissolved oxygen occurrences (< 2 mg / L) observed in Upper Klamath Lake during blue-green algal blooms. Motile aeromonad bacteria were consistently isolated from asymptomatic fish and few external parasites were observed on the fish. Flow interruption, due to bacterial slime and algal solids, was a persistent problem in this pilot effort and indicates that future disease studies employ in-situ live-cages rather pumped aquaria systems.

Background- Both Lost River Sucker (*Deltistes luxatus*) and Snortnose Sucker (*Chasmistes brevirostris*) of Upper Klamath Lake, Oregon are listed as endangered species (US Fish and Wildlife Service, 1988). Low survival of fry (0+) through their first year, as well as occasional adult fish kills, has been identified as a chronic problem affecting population numbers. Upper Klamath Lake is a highly eutrophic system with extensive cyanobacterium (*Aphanizomenon flos-aquae)* blooms during the summer. During the bloom cycle water quality can be adverse to fish health (i.e. pH> 9.5, low dissolved oxygen, and high ammonia). Health surveys of adult and juvenile suckers have detected infections by a number of external parasites and bacteria in both moribund fish and those with normal appearance (Foott 2004). This study examined the relationship between adverse water quality and changes in the severity and composition of fish pathogens. This pilot effort had four objectives:

1. Determine whether bacterial and /or parasitic infection of 0+ suckers become more severe or change in pathogen composition after exposure to Upper Klamath Lake water (A-canal) during a blue-green algal bloom. Compare sentinel fish health to the donor population that was reared outside of the lake.
2. Document mortality and /or morbidity associated with infectious disease in the sentinel population and its relationship with duration of exposure to lake water.
3. Document water quality parameters (temperature, dissolved oxygen, pH, and ammonia) during exposure period.
4. Determine logistics and feasibility for a large-scale study

Methods: On 13August 2007, 150 fry were moved from the Barnes ranch aquaculture facility near Klamath Falls to 40 L replicate aquaria at the USBR A-canal fish examination station (see cover photo). These fry, of unknown sucker species composition, had been captured in June as 10 – 20 mm larvae in the lower Williamson River (K. Russell, USFWS pers. comm.). One month prior to the movement, this population was screened for viral and parasitic infection. No

virus was isolated in 8 (5 fish pools) inoculated onto EPC, FHM (26°C) and CHSE214 cell lines. The parasites *Ichthyobodo sp.* and *Ichthyophthirius multifiliis* were observed in wet mount preparations from several fish while histological examination of gill, kidney, liver, and intestinal tract revealed no abnormalities or parasites. Fish were not fed during the experiment and aquaria were checked once per day for mortality and flow (approximately 0.5 gpm).

A total of 30 fish were sampled for the assays listed below from 2 aquaria at 0 (sampled at Barne's facility immediately prior to transfer to A-canal), 7, 14, and 22 days post-exposure (dpe). All fish were euthanized by an overdose of MS222 and measured for standard length. A skin scraping and a dissected gill filament wet mount from 12 fish was examined by phase microscopy (10-40x). Twenty fish were sampled for internal bacteria by first spraying the skin with 70% isopropyl alcohol, opening the peritoneum with sterile scissors, inserting a 1µL sterile loop into the kidney, and streaking onto TYG agar (2g tryptone, 0.5 g yeast extract, 3g gelatin, 15g agar, 1L deionized water). Plates were incubated at 25°C and colonies evaluated at 24h using standard diagnostic methods (Thoesen 1994). A total of 72 fish used for the bacterial and wet mount examination were stored in isopropyl alcohol for later vertebral counts by X-ray analysis. Lip morphology and vertebral counts were used to speciate the collection group. Ten fish were fixed in Davidson's fixative for 48h, processed for 5µm sagittal paraffin sections, stained with hematoxylin and eosin, and microscopically examined at 10 and 40x magnification (Humason 1979).

A YSI 600 XLM datasonde was placed into the head box of the aquaria system and recorded specific conductance (µmhos / cm), oxygen (mg/L), and pH each hour. A calibrated probe exchange occurred weekly. Onset temperature probes recorded temperature in 2 aquaria. The following water quality assays (spot checks) were conducted in 2 aquaria as described on each sample days:

1. pH	Beckman 12 meter
2. Total ammonia nitrogen (TAN)	Hach DR850 meter, method 8155

Unionized ammonia concentration derived from temperature, TAN, and pH tables in Boyd (1979).

3. Dissolved oxygen	Hach HQ10 LDO oxygen meter
4. Alkalinity	LaMotte WAT-DR 4491 kit
5. Nitrate-N	LaMotte PLN-DR 7421 kit

Results:

Water quality –Fish experienced a 9.2°C increase (Barne's ranch facility 12.7°C, A-canal 21.9°C) upon transfer to the A-canal aquaria on 13 August. Mean daily temperature ranged from 20.2 ° to 22.6°C over the 22 d exposure (Fig. 1). Mean daily pH of the aquaria water supply (YSI datasone) ranged from 7.36 to 9.38 with a maximum hourly value of 9.62. There were two significant declines linked with cessation of flow in the aquaria (Fig. 2). Specific conductance varied inversely with the pH during the flow cessation events (Fig.2). During cleaning operations of the head tank a sulfide smell was detected however no tests for hydrogen sulfide were done. It is likely that bottom sediment within the head tank became anaerobic for portions for the 22 d experiment. Mean daily dissolved oxygen of the aquaria water supply (YSI datasone) ranged 0.30 – 6.3 mg / L with the extremely low values associated with cessation of flow (Fig. 3). Spot checks of temperature, pH, dissolved oxygen, and ammonia in aquaria showed large discrepancies in oxygen measurements from the YSI datasone located in the head tank (Table 1). The YSI datasone dissolved oxygen concentrations were 1 – 2 mg / L lower than the spot checks taken during the same hour. Despite these differences, oxygen values were considered low (2 -3 mg / L for 7,14, and 22 dpe). The pH measurements were similar between the spot checks and YSI datasone (< 0.2 units). Weekly unionized ammonia concentration ranged from 0.034 to 0.102 mg / L (Fig. 4). A water sample collected from A-canal on 04Sept. (UIA 0.044 mg / L) was similar to the aquaria (UIA 0.034 mg / L). Nitrite-nitrogen was below the detection limit of kit (0.1 mg/L) and alkalinity ranged from 52 – 56 mg $CaCO_3$ mg / L at A-canal and was 162 mg / L at Barnes ranch on 13August.

Figure 1. Mean daily temperature (°C) in A-canal aquaria from 13August to 04September.

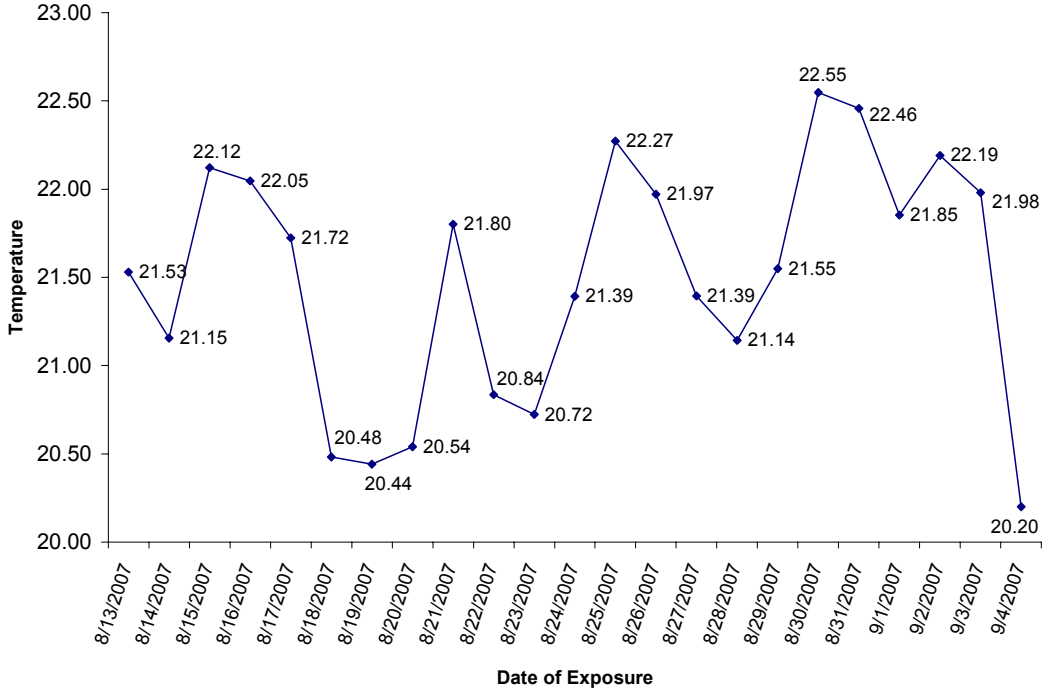

Figure 2. Mean daily pH and specific conductance (µmhos/ cm) recorded by YSI datasone in source water for A-canal aquaria

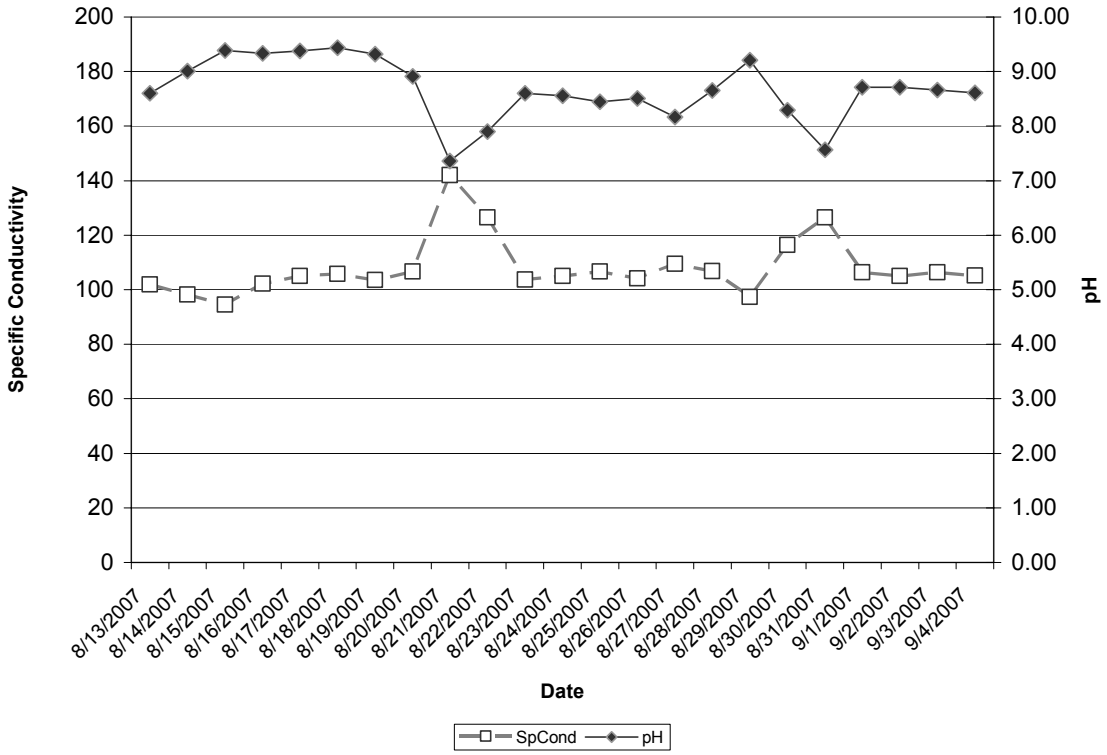

Figure 3. Mean daily dissolved oxygen concentrations (mg / L) recorded by YSI datasone in source water for A-canal aquaria

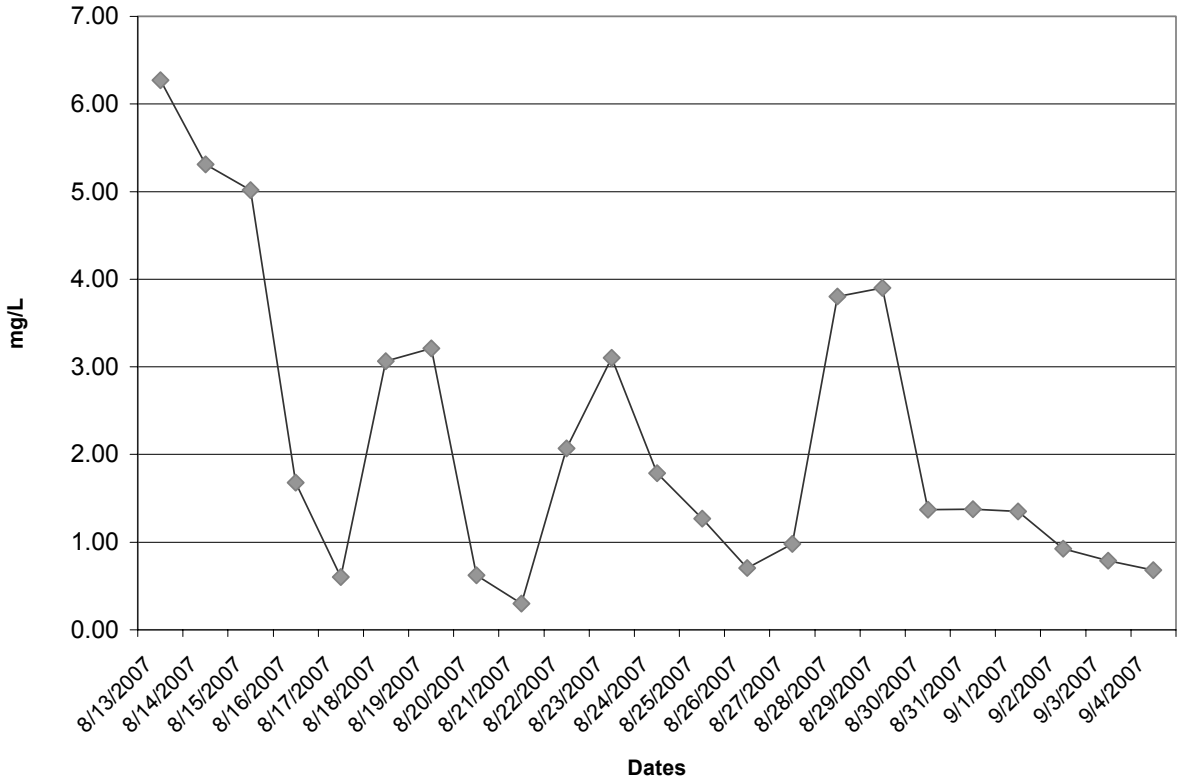

Table 1. Temperature (°C), pH, dissolved oxygen (mg /L) and unionized ammonia measurements taken on sample dates at 09:00 (dpe). Values reflect maximum value recorded from 1- 3 tanks.

Date (dpe)	°C	pH	DO	UIA
13Aug (0)				Not done*
Barnes ranch	12.7	8.21	8.0	
tanks	21.9	9.47	5.4	
20Aug (7)	19.1	9.22	3.3	0.102
27Aug (14)	19.6	8.52	3.3	0.037
04Sep (22)				
Tanks	19.9	8.51	2.0	0.044
A-canal	20.6	8.97	4.8	0.034

* meter malfunction

Sucker mortality, species composition and length- A moderate increase in mortality with signs of external parasitism (flashing) occurred a week prior to the 13Aug move to A-canal (R. Barnes personnel communication). These issues were resolved after treatment with 0.5 ppt salt, 25 ppm formalin, and oxytetracycline (feed). A total of 10 mortalities were recorded over the 22 d exposure at A-canal. Our estimate of 7% cumulative percent mortality was calculated:

Population of 145 = 10 mortality+ 93 sampled at A-canal+42 survivors on day22
7% cum. Mort. =10 mortality / 145 population

The dense algal content of the aquaria made observation of fish difficult and likely resulted in missed mortality records. As determined by vertebral counts, species composition of the exposed population was 79% Shortnosed sucker (SNS), 7% Lost River Sucker (LRS), 3% undetermined and 11% Klamath Large-scale Sucker. Mean standard length ranged from 27 – 30 mm over the three week exposure (Table 2). The 22 dpe group was significantly larger than the 0 dpe fish (Kruskal – Wallis 1-way ANOVA on ranks, H = 9.886, 3 df, P=0.02, Dunn's multiple comparison method) however there was no difference between 7, 14, and 22 dpe groups indicating little to no growth over the exposure.

Table 2. Mean standard length (Std Dev.) of sucker fry sampled at 0, 7, 14, and 22 dpe to A-canal water.

Date (dpe)	Standard Length (mm)
13Aug (0)	27 (5)
20Aug (7)	29 (4)
27Aug (14)	28 (5)
04Sep (22)	30 (4)

Wet mount observations –Composition of external parasites changed over the 22 day study however the severity of infection remained mild (Table 3). At 7 dpe, the fish had one or more sessiline peritrich species (branched form presumptively identified as *Epistylus sp*. and single forms presumptively identified as either *Apiosoma* or *Ambiphyra sp.*) as well as the flagellate *Ichthyobodo* (previous genus name *Costia*). These parasites were not detected in fish examined at later dates. The ciliate parasite *Trichodina* was observed on 1 fish from both the 14 and 22 dpe samples. Gill morphology was deemed normal at all sample dates.

Table 3. Prevalence of external parasite infection (%) observed by microscopic examination of skin and gill. Twelve fish were sampled on each collection date.

	13AUG		20AUG		27AUG		04SEP	
Parasite	Skin	Gill	Skin	Gill	Skin	Gill	Skin	Gill
Ichthyobodo	0	0	17	0	0	0	0	0
Trichodina	0	0	0	0	0	8	8	0
Ichthyopthirius multifiliis	0	8	0	0	0	0	0	0
Epistylus / Ambiphrya	0	0	83	25	0	0	0	0

Bacterial isolation- Motile aeromonad bacteria (MA = motile gram-negative rods that are cytochrome oxidase positive) were isolated in fry prior to and after exposure at A-canal (Table 4).. We performed additional assays (Oxidative-Fermentative, Triple Sugar iron, API 20E panel) on 3 to 5 MA isolates per collection and presumptively identified them as *Aeromonas hydrophilia.* A low number of gram-positive cocci (catalase positive *Staphylococcus* group) were also isolated from the fish. The lack of bacterial isolation from the 7 dpe group is likely related to shipment temperatures to the Leetown lab. None of the sampled fish showed signs of bacterial infection such as hemorrhagic foci within internal organs or on the skin.

Table 4. Prevalence of motile aeromonad bacteria isolations from internal samples collected from sucker fry. Recorded as positive / total (%).

Date (dpe)	Positive / total (%)
13Aug (0)	6 / 12 (50%)
20Aug (7)	0 / 20 (0%)**
27Aug (14)	17 / 20 (85%)
04Sep (22)	10 / 20 (50%)

** Plates shipped to USGS Leetown lab

Histological examination- Edema and moderate hyperplasia of gill epithelium was observed in all fry collected on 13Aug (0 dpe) at the Barne's facility. Bath treatments (formalin and salt) may have influenced this occurrence. One fish had *Myxobolus sp.* spores within the caudal musculature however there were no signs of associated inflammation or lesions. Hepatocytes had extensive vacuoles presumptively containing glycogen (Fig 4). *Ichthyophthirius multifiliis* was observed embedded under the skin and gill epidermis of 1 suckers from the Barne's facility. Sections from 7 dpe fry had increased numbers of eosinophilic granular cells (EGC) in the peritoneal adipose tissue and Leydig (alarm) cells in the dermis (Fig 5) when compared with 0 dpe fish. These fish also showed a

reduction in gill edema and hepatocyte glycogen content (Fig. 4). An unidentified peritrich protozoan was observed on the skin of two 7 dpe fish. In fish collected at both 14 and 22 dpe, there was marked hypertrophy of cells between gill lamellae (presumptive chloride cells). No parasites were seen in the 14 and 22 dpe fish and no abnormalities were seen in any other organs.

Discussion:

Water quality in the aquaria was generally worse than the lake due to flow interruptions. Despite the 22 day exposure to adverse water quality, juvenile suckers did not incur significant mortality or an infectious disease epizootic. Both dissolved oxygen and unionized ammonia levels were at levels approaching LC50 values reported for juvenile LRS and SNS. If the head tank YSI datasone measurements are reflective of the 40L aquaria, dissolved oxygen values were less than 1.0 mg / L for extend periods during the exposure. These values are lower than the oxygen LC50 (1.34 and 2.10 mg / L) for juvenile SNS and LRS reported by Saiki et al. 1999. In the current challenge, fish had access to the water surface to facilitate air "gulpping" unlike the challenge system used by Saiki et al (1999). The discrepancies observed between spot check oxygen data and the continuous YSI datasone suggests that probe may have become fouled in the head tank between weekly exchanges or that stagnant water immediately adjacent to the probe influenced the measurements. Despite these questions, it is apparent that dissolved oxygen was limited for extended portions of the 22 d experiment. The pH levels (7.36 – 9.38 mean daily pH) recorded in this study were relatively mild for Upper Klamath Lake.

We did not observe signs of disease due to either bacteria or external parasites. Bacterial flora collected from the fish's interior (peritoneum / kidney) was consistently dominated by motile aeromonad bacteria however external parasite flora did changed in composition. Previous investigations of Upper Klamath Lake fish have documented similar parasite and bacterial infections in both moribund and asymptomatic fish (Foott 2004). In a 2004 National Wild Fish Survey collection of 325 Tui chubs and 389 Fathead minnows from the lake, similar external parasites (53% *Trichodina, 13% Epistylus,* and 67% *Ichthyobodo* incidence of infections) and bacteria (3- 22% *Aeromonas hydrophilia* incidence of infection) were recorded.

The decrease in hepatocyte glycogen vacuoles and lack of growth indicates the suckers were food deprived. While we observed chronomid larvae and *Daphina* within aquaria no feeding was performed over the 22 d exposure. Despite the elevated ammonia levels we did not observed significant abnormalities in gill lamellae by light microscopy. The unionized ammonia LC50 value of 1.06 mg /L reported by Saiki et al. (1999) was similar to the 20Aug spot check measurement of 1.02 mg / L. Lease et al. (2003) report that lamellar thickness and diffusion distance of juvenile Lost River suckers increased with elevated ammonia concentrations (0.01 – 0.37 mg NH_3- N / L). These researchers examined thin sections (plastic resin 2-3µm sections) of gill in comparison to the 5- 6 µm paraffin embedded sections of this study. The thinner sections provide for better evaluation of minute changes in tissue dimensions than paraffin sections used in this study.

Concerning the original 5 objectives of this pilot study:

1. We did not observe disease (infection or significant lesions) or significant mortality in sucker fry exposed for 22 days to adverse water quality that was comparable to extreme levels of ammonia and dissolved oxygen observed in Upper Klamath Lake during blue-green algal blooms.
2. Blockage of water lines with bacterial film and algae dictates that future exposure studies are conducted in-situ (live cages with daily cleaning of mesh).

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Figure. 4 Hepatocytes of juvenile suckers collected at Barnes facility (top) showing vacuolization suggestive of glycogen content and from the A-canal aquaria at 7 dpe (bottom) that are absent of vacuoles (40x magnification H&E).

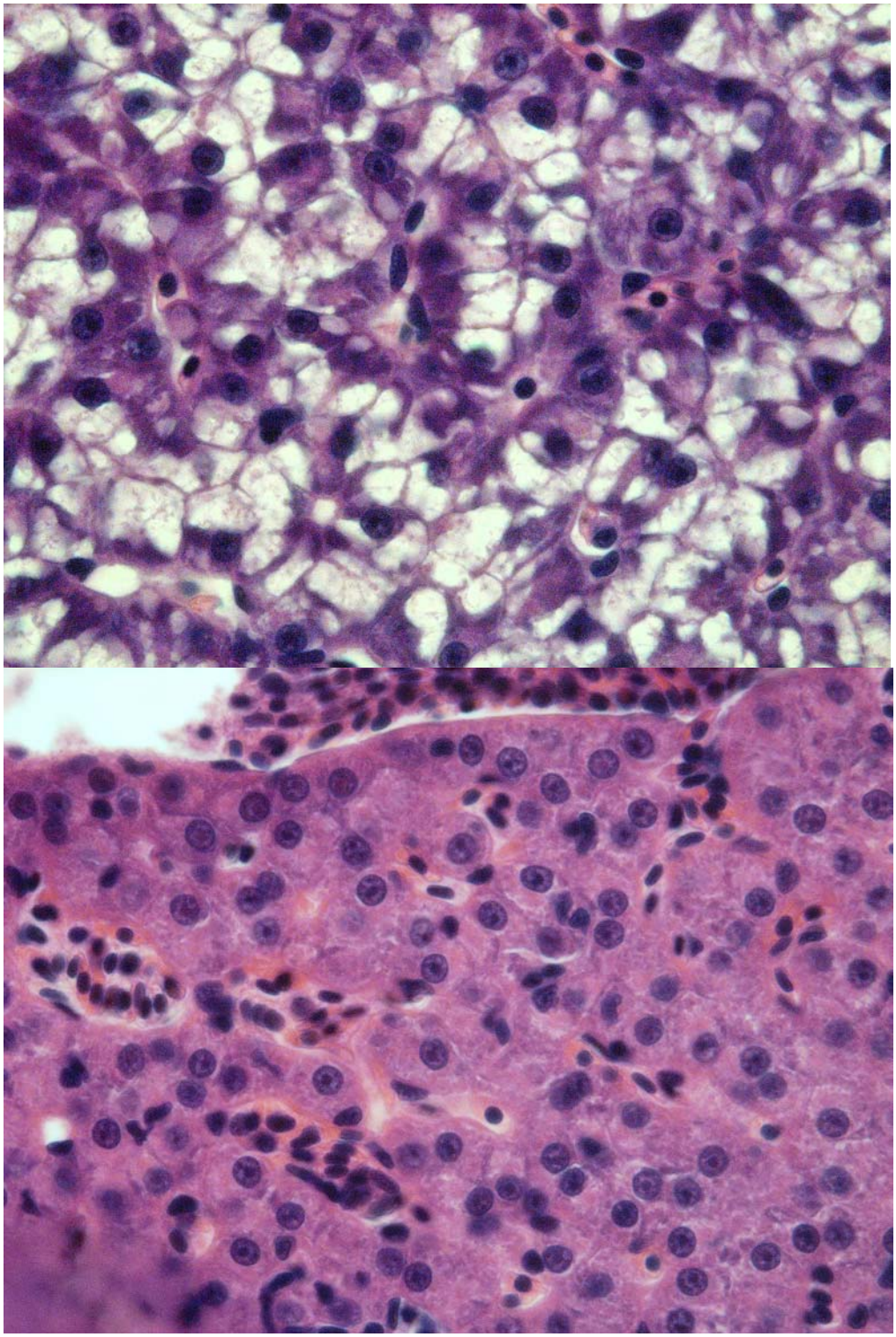

Figure 5. Leydig cells (arrow) in dermis of juvenile sucker held at A-canal (40x magnification H&E).